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TOPICAL REVIEW

Neurological channelopathies: new insights into disease mechanisms and ion channel function

Dimitri M. Kullmann[1] and Stephen G. Waxman[2]

[1] *Institute of Neurology, University College London, London, UK*
[2] *Department of Neurology and Center for Neuroscience & Regeneration Research, Yale University School of Medicine, New Haven, CT, USA*

Inherited mutations of ion channels provide unique insights into the mechanisms of many neurological diseases. However, they also provide a wealth of new information on the fundamental biology of ion channels and on neuron and muscle function. Ion channel genes are continuing to be discovered by positional cloning of disease loci. And some mutations provide unique tools to manipulate signalling cascades, which cannot be achieved by pharmacological intervention. Here we highlight some unanswered questions, and some promising areas for research that will likely lead to a fuller understanding of the link from molecular lesion to disease.

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Corresponding author D. M. Kullmann: UCL Institute of Neurology, Department of Clinical Neurology, Queen Square, London WC1N 3BG, UK. Email: d.kullmann@ion.ucl.ac.uk

Neurological channelopathies in 2010: a snapshot

Although the term 'channelopathy' was coined less than 20 years ago, inherited mutations of ion channels are now recognised to affect a wide range of organs and to represent a substantial disease burden. An increasing number of neurological channelopathies have been identified and characterized, and have begun to teach us important lessons about the molecules and cellular processes that underlie electrical excitability and disorders of excitability. This Special Issue of *The Journal of Physiology* presents reviews of recent research in muscle, nerve and brain channelopathies, focusing on areas where there have been particularly important advances. In introducing this Special Issue, rather than providing a systematic review of the history of channelopathy research, we highlight some of the emerging principles, and point to especially promising areas of research for the next decade. Comprehensive reviews of the neurological channelopathies from the point of view of the different families of ion channels are available elsewhere (Dib-Hajj *et al.* 2010; Kullmann, 2010). Nevertheless, the following points capture some of the progress made since the first ion channel mutations were identified in association with monogenic neurological disease.

(i) Mutations have been identified in at least 29 different ion channel genes (Table 1), not counting reports of sporadic variants of possible pathological impact that are increasingly appearing in the literature.

(ii) Mutations associated with monogenic disease affect almost all ion channel families: voltage-gated potassium, calcium and sodium channels, inwardly rectifying potassium channels, chloride channels, and glycine, GABA and nicotinic acetylcholine receptors. Mutations have also been identified in connexins and intracellular channels. The only major class of neuronal ion channels where mutations have not been reported in association with neurological disease is the ionotropic glutamate receptors. The explanation for this omission remains unclear at this time.

(iii) Although many mutations result in loss of function, some missense mutations result either in an increase in ion flux (often by impairing inactivation, but in some cases by enhancing activation) or in a gain of abnormal function. Some of these experiments of nature have shed unexpected new light on ion channel biophysics and on the fundamental biology of excitable tissues.

(iv) Of the monogenic 'pure' epilepsies (that is, where epilepsy is the major or only manifestation of the genetic lesion), almost all the genes are ion channels. Indeed, several hundred *de novo* mutations of a single gene (*SCN1A*, encoding the α1 subunit of sodium channels) have already been identified, mainly in children with an especially severe form of epilepsy, severe myoclonic epilepsy of infancy or SMEI (Catterall *et al.* 2010; Meisler *et al.* 2010).

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Table 1. The neurological channelopathies

		Gene	Channel subunit	Disease
CNS	Sodium channels	*SCN1A*	α subunit of Nav1.1	Epilepsy, migraine
		SCN1B	β1	Epilepsy
		SCN2A	α subunit of Nav1.2	Epilepsy
	Potassium channels	*KCNQ2*	Kv7.2	Epilepsy
		KCNQ3	Kv7.3	Epilepsy
		KCNMA1	BK	Epilepsy with dyskinesia
		KCNA1	Kv1.1	Episodic ataxia
		KCNC3	Kv3.3	Ataxia
	Calcium channels	*CACNA1H*	α1H subunit of Cav3.2	Epilepsy
		CACNA1A	α1A subunit of Cav2.1	Episodic or progressive ataxia, migraine, epilepsy
	$GABA_A$ receptors	*GABRA1*	α1	Epilepsy
		GABRB3	β3	Epilepsy
		GABRG2	γ2	Epilepsy
	Nicotinic ACh receptors	*CHRNA2*	α2	Epilepsy
		CHNRA4	α4	Epilepsy
		CHRNB2	β2	Epilepsy
	Glycine receptors	*GLRA1*	α1	Hyperekplexia
		GLRB	β	Hyperekplexia
Peripheral nerve	Sodium channel	*SCN9A*	α subunit of Nav1.7	Excessive pain, insensitivity to pain
Muscle	Sodium channel	*SCN4A*	α subunit of Nav1.4	Periodic paralysis, myotonia
	Potassium channels	*KCNJ2*	Kir2.1	Periodic paralysis
		KCNJ18	Kir2.6	Periodic paralysis
	Calcium channel	*CACNA1S*	α1S subunit of CaV1.1	Periodic paralysis
	Chloride channel	*CLCN1*	CLC-1	Myotonia
	Nicotinic ACh receptors	*CHRNA1*	α1	Congenital myasthenic syndromes
		CHRNB1	β1	Congenital myasthenic syndromes
		CHRNG	γ	Congenital myasthenic syndromes
		CHRND	δ	Congenital myasthenic syndromes
		CHRNE	ε	Congenital myasthenic syndromes

(v) Similarly, several monogenic pain syndromes (inherited erythromelalgia, paroxysmal extreme pain disorder) have thus far been linked to mutations in *SCN9A*.

(vi) Channelopathies affect almost all areas of neurological practice, including epileptology (Wimmer *et al*. 2010; Catterall *et al*. 2010; MacDonald *et al*. 2010; Meisler *et al*. 2010), movement disorders (Rajakulendran *et al*. 2010), headache (Pietrobon, 2010), peripheral nerve, pain (Cregg *et al*. 2010; Estacion *et al*. 2010), and myology (Cannon, 2010; Matthews & Hanna, 2010) (Table 1).

A genetic conundrum

Despite the overwhelming evidence that ion channel disorders underpin many forms of neurological disease, in only rare cases does a clinical diagnosis lead to a high expectation of identifying a mutation (notwithstanding the practical obstacles to gene sequencing). Such disorders include the inherited disturbances of muscle membrane excitability (myotonias and different forms of periodic paralysis), certain forms of epilepsy including SMEI, excessive startle disorder (hyperekplexia), the episodic ataxias, and some inherited pain syndromes (inherited erythromelalgia, paroxysmal extreme pain disorder). With the exception of the chloride channel myotonias, hyperekplaxia and SMEI (where recessive inheritance, compound heterozygosity or *de novo* mutations often occur), most patients in whom a monogenic channelopathy can be identified have a positive family history, because the disease is transmitted as a dominant trait. Far more common are patients with acquired or so-called 'idiopathic' diseases. Migraine and several forms of idiopathic generalised epilepsy (IGE), typically associated with spike-and-wave EEG, exhibit high heritability: it has been estimated that about 70% of the individual's risk of developing either disorder is accounted for by genetic factors. And yet these diseases very rarely exhibit Mendelian inheritance, implying that they arise from the interplay of multiple genes. A variant in any one of these genes may not be sufficient to determine whether an individual develops a disease,

but in interaction with other inherited gene variants, it may tip the balance. Although these susceptibility genes remain to be identified, the best candidates are those that encode ion channels. This is precisely because, of the rare monogenic forms of migraine (familial hemiplegic migraine) or epilepsy (various forms of IGE, as well as other localisation-related syndromes and childhood seizure disorders), most of the causative genes are ion channels.

Given that whole genome scans are now routine, it is disappointing that the common genetic susceptibility variants have not been identified for idiopathic neurological diseases such as migraine (which affects over 10% of the Western population) and epilepsy (which affects 0.5%), and in subjects with a low threshold for, or chronic, pain. After all, such variants are increasingly being identified in other common diseases such as diabetes mellitus, and apolipoprotein E4 is strongly associated with Alzheimer's disease. A possible explanation is that many individually rare variants in many (presumably ion channel) genes underlie the genetics of such neurological diseases. Identifying such genetic variants, and separating them from neutral polymorphisms (or even variants that reduce the chance of developing a disease) is a major data mining challenge. Nevertheless, it may be possible to stratify genetic variants identified in individuals suffering from a suspected polygenic channelopathy by functional expression *in vitro*. Indeed, ion channels are uniquely amenable to detailed biophysical analysis. Such an approach potentially allows one to ask whether those variants that cause loss- or gain-of-function are, as a whole, disproportionately over- or under-represented among affected individuals. Rajakulendran *et al*. (2010) have applied precisely this approach to show that loss-of-function variants of a calcium channel gene (*CACNA1A*) are over-represented among sporadic patients with a combination of episodic ataxia and epilepsy, providing compelling evidence implicating this gene as a susceptibility factor.

Why do so many channelopathies have paroxysmal manifestations?

Epilepsy, migraine, episodic ataxia, periodic paralysis, inherited erythromelalgia and paroxysmal extreme pain disorder all share in common a disease course characterised by normal neurological development and function punctuated by attacks of disabling symptoms. Why? And given that the molecular lesion is constant, how does the nervous system compensate in between attacks? Indeed, this is puzzling, because neurological disorders caused by mutations of genes other than ion channels often lead to abnormal brain development, mental retardation, or fixed or progressive motor disorders. A possible explanation is that neuronal excitability is a tightly regulated parameter and that alterations in the expression level or function of one channel can lead to compensatory changes in other ion channels. Such changes have been observed in neurons in culture exposed to experimental manipulation of excitability (Turrigiano, 2008), or following genetic deletion of ion channel subunits in the cerebellum (Brickley *et al.* 2001). Nevertheless, normal function is vulnerable to various stressors in patients with channelopathies, implying a smaller safety factor before a paroxysm is triggered.

In some of these disorders, a reasonable explanation can be proposed for the development of the ictus, although it is not always clear why certain precipitants trigger them. In hyperkalaemic periodic paralysis, impaired fast inactivation of sodium channels provides for a positive feedback loop, where delayed repolarization of the muscle membrane potential leads to a further increase in extracellular potassium. There are also reasonably compelling mechanistic accounts for seizure propagation and for the aura of migraine, in one case mediated by abnormal recruitment of neuronal circuits and in the other by an advancing wave of cortical depolarization. However, why the episodic ataxias affect entire cerebellar circuits at once remains unclear. Among recent breakthroughs is the improved understanding of hypokalaemic periodic paralysis. This dominantly inherited disorder is uniformly caused by missense mutations that replace positively charged arginine residues in the S4 segments of either the sodium channel $Na_V1.4$ or the calcium channel $Ca_V1.1$, which act as transmembrane voltage sensors (Matthews & Hanna, 2010). Until recently the mutations were thought to result in a relative loss of function, but how this predisposed to depolarization and muscle inexcitability under conditions of hypokalaemia was not understood. It emerges that replacement of the arginine residues by other amino acids leads to a cation leak that bypasses the main ion conducting pore of the channel.

Why is clinical onset in some channelopathies delayed?

Individuals affected by inherited channelopathies carry the mutant gene, and presumably produce the mutant protein or fail to produce a normal complement of the normal protein, beginning *in utero*. Consistent with this, clinical abnormalities are often apparent beginning in infancy. Yet in some families with inherited channelopathies, clinical onset is delayed until late childhood, adolescence, or adulthood. What are the mechanisms underlying this time-dependent onset of clinical disease? A clue may come from studies of families with late-onset inherited erythromelalgia. In some of these families there are

maturational changes in splice isoforms of the $Na_V1.7$ channel, with the mutation shifting voltage-dependence in the adult, but not juvenile, splice variant (Choi *et al.* 2010). $Na_V1.7$ mutant channels in other families with late-onset erythromelalgia studied to date display a hyperpolarizing shift in the voltage dependence of activation, as is seen for mutant $Na_V1.7$ channels linked to early-onset inherited erythromelalgia. But the magnitude of the hyperpolarizing shift in activation is smaller in these late-onset families (Cheng *et al.* 2008; Han *et al.* 2009). And at the level of cellular function, the late-onset mutant channels induce hyperexcitability in dorsal root ganglion neurons, but the increase in excitability is smaller than that produced by mutations linked to early-onset erythromelalgia. This suggests that time-dependent presentation of disease signs and symptoms may in some kindreds reflect the presence of compensatory mechanisms, early in life, which suppress clinical manifestations. According to this model, later in life, the protective effects of these compensatory mechanisms are overcome, due either to cumulative events, or to maturational switching of splice isoforms, or of modifier genes and their products. Whether the putative protective mechanisms are molecular, i.e. reflect compensatory changes in expression of other channels which act to maintain excitability at close-to-normal levels, or operate at the systems levels, e.g. by processes such as central gating, remains to be elucidated. Detailed understanding of the mechanistic basis for time-dependent onset of clinical disease, and of the protective mechanisms that contribute to it, may ultimately provide a basis for therapeutic interventions that act on, or via, these mechanisms, to suppress clinical signs and symptoms in patients with idiopathic disorders.

Channelopathies are leading to fundamental advances in ion channel research

Several channels were discovered by positional cloning in families with unusual disorders, including the potassium channels Kv7.2 and Kv7.3 in benign familial neonatal convulsions. The subsequent discovery that they co-assemble to form a slowly activated potassium channel solved a long-standing puzzle about the molecular identity of the 'M' current, which is attenuated upon activation of muscarinic receptors in many neurons. New ion channel genes continue to be discovered, most recently *KCNJ18*, which encodes an inward rectifying channel in skeletal muscle, mutations of which predispose to periodic paralysis in patients with hyperthyroidism (Ryan *et al.* 2010).

Close attention to the biophysical consequences of inherited mutations has also led to the identification of potentially powerful tools to manipulate signalling cascades. Thus, the different *CACNA1A* mutations underlying familial hemiplegic migraine lead to an increase in the function of individual $Ca_V2.1$ channels, in some cases by shifting the voltage threshold of activation to more negative potentials (Pietrobon 2010). Because exocytosis at central synapses is triggered by calcium influx via very few channels, these mutations may provide unique new tools to investigate how individual channels interact.

The still-expanding universe of channelopathies promises to provide additional clues about ion channels, how they function, and how their abnormal function can produce disease. While, at first blush, it might seem that physiological assessment of the rapidly expanding list of channelopathies identified by molecular or genetic analysis might be beyond reach, robotic patch-clamp methods may facilitate screening of some of the important biophysical parameters of the mutant channels (Estacion *et al*. 2010). Hands-on physiologists need not, however, retreat. As can be seen by a comparison of robotic assessment (Estacion *et al*. 2010) and in-depth analysis of mutant channels causing the same disease by human physiologists (Rush *et al.* 2006), there will always be a need for study of channelopathies by incisive investigators. That analysis will undoubtedly be forthcoming over the coming decade, and we can with confidence predict that channelopathies will continue to teach us important lessons.

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